

Supporting Information

Mitsunobu mischief: Neighbor-directed histidine N(τ)-alkylation provides access to peptides containing selectively functionalized imidazolium heterocycles

Wen-Jian Qian and Terrence R. Burke, Jr.*

¹Chemical Biology Laboratory, Center for Cancer Research, National Institutes of Health, NCI at Frederick, Frederick, MD 21702, U. S. A

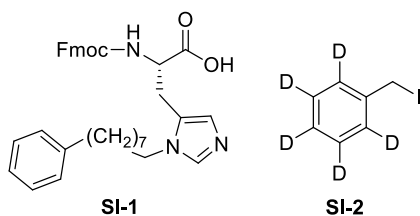
*To whom correspondence should be addressed

E-mail: burkete@helix.nih.gov

Table of Contents	Pg
1. General Synthetic	S4
2. Synthesis of Non-natural Fmoc-Protected Amino Acids	
2.1 Fmoc-homocysteic Acid (SI-3)	S5
2.2.1 Fmoc-His[N(π)-(CH₂)₈Ph]-OAllyl (SI-4)	S5
2.2.2 Fmoc-His(N(π)-(CH₂)₈Ph, N(τ)-(CH₂)₂Ot-Bu)-OH (SI-5)	S7
2.3 Fmoc-His(N(π)-(CH₂)₈Ph, N(τ)-d₅-Bn)-OH (SI-6)	S7
3. Role of the pThr Residue in N(τ)-Alkylation	
3.1 General Peptide Synthesis Procedures	S7
3.2 On-resin Mitsunobu Reactions and Analytical HPLC- Mass Spec.....	S7
3.3 Role of Acidic Hydroxyl: Synthesis of Peptide SI-8	S8
4. Non-involvement of Phosphoryl Ester Transfer	S10
5. Accommodation of Spatial Differences Between pThr and His Residues	11
6. Promotion of N(τ)-Alkylation by Residues other than pThr	
6.1 General Synthesis of Peptides for Table S6	S13
6.2 Synthesis of Resin-bound Peptide 18b	S13
6.3 Peptide 19b Structural Verification	S13
6.4 Peptide 20b Structural Verification	S14
6.5 Peptide 20c 20b Structural Verification	S14
7. Inhibition of Mitsunobu Esterification by a Proximal pThr[(OBnO)(OH)] Residue	S16
8. Mass Spectral Characterization	S17
9. References	S20
10. ¹H-NMR and ¹³C-NMR Spectra for SI-3 – SI-6.....	S21

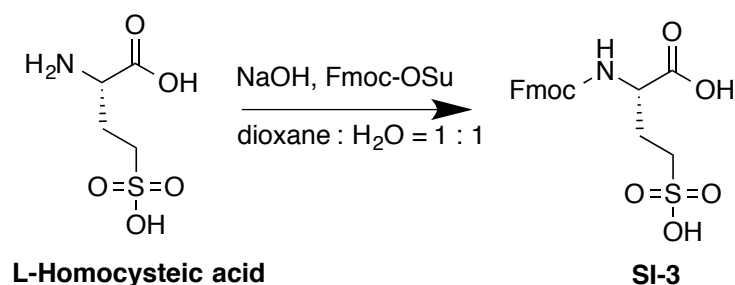
1. General Synthetic.

All experiments involving moisture-sensitive compounds were conducted under anhydrous conditions (positive argon pressure) using standard syringe, cannula, and septa apparatus. All solvents were purchased in anhydrous form (Aldrich) and used directly. HPLC-grade hexanes, EtOAc, CH₂Cl₂, and MeOH, were used in chromatography. NovaSyn®TGR resin and NovaSyn®TG Siber resin were employed using standard Fmoc-protected amino acids, Fmoc-Thr(PO(OBzl)OH)-OH, Fmoc-Tyr(PO(OBzl)OH)-OH, Fmoc-Ser(PO(OBzl)OH)-OH, Fmoc-D-Thr(PO(OBzl)OH)-OH, Fmoc-Ser(Trt)-OH, Fmoc-Leu-OH, Fmoc-Pro-OH and Fmoc-Glu(OAll)-OH, which were purchased from Novabiochem. 4-Penten-1-ol, Benzyl-2,3,4,5,6-d₅ alcohol, allyl alcohol, L-Homocysteic acid, triphenylphosphine and diethyl azodicarboxylate (DEAD) were obtained from Aldrich. The 8-phenyl-1-octanol was obtained from Alfa Aesar. Fmoc-His[N(π)-(CH₂)₈Ph]-OH (**SI-1**)¹ and Benzyl-2, 3, 4, 5, 6-d₅ iodide (**SI-2**)² were synthesized according to the indicated literature procedures. Analytical TLCs were performed using Analtech pre-coated plates (Uniplate, silica gel GHLF, 250 nm) containing a fluorescence indicator. NMR spectra were recorded using a Varian Inova 400 MHz spectrometer, with coupling constants being reported in Hertz, and peak shifts being reported in δ (ppm) relative to TMS. Nominal mass spectra (ESI) were measured with an Agilent 260 1200 LC/MSD-SL system. High resolution mass spectra (HRMS) for confirmation of elemental compositions were obtained by positive ion, ESI analysis on a Thermo Scientific LTQ-XL Orbitrap mass spectrometer with HPLC sample introduction using a short narrow-bore C₁₈ reversed-phase column with CH₃CN - H₂O gradients. Reported m/z values are the average of eight or more scans over the chromatographic peak of interest.



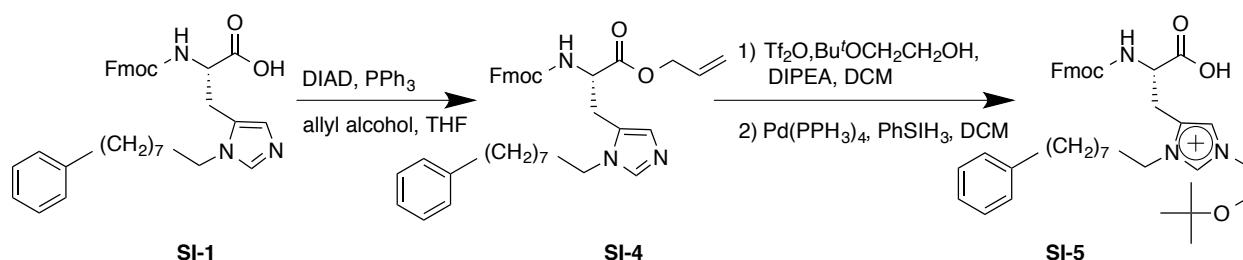
2. Synthesis of Non-coded Fmoc-Protected Amino Acids

2.1 Fmoc-homocysteic Acid (SI-3).



L-Homocysteic acid (100 mg, 0.55 mmol) and NaOH (55 mg, 1.36 mmol) were dissolved in H₂O (5.5 mL) cooled in an ice bath. A solution of Fmoc-OSu (200 mg, 0.6 mmol) in dioxane (5.5 mL) was added in one portion. The mixture was stirred at room temperature (4 h) and then the mixture was evaporated under reduce pressure and diluted with H₂O (10.9 mL). The aqueous solution was washed with diethyl ether and acidified (37% aqueous HCl) and the aqueous solution was lyophilized and purified (CombiFlash with a C₁₈ column using MeCN and H₂O) to provide **SI-3** 0.15 g (68%). [α]_D²⁰ 3.4 (c 0.45, CHCl₃ : MeOH = 10 : 1); ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.89 (d, *J* = 8.0 Hz, 2H), 7.82 (d, *J* = 8.0 Hz, 1H), 7.74 (d, *J* = 8.0 Hz, 2H), 7.42 (t, *J* = 8.0 Hz, 2H), 7.37 – 7.30 (m, 2H), 4.30 – 4.16 (m, 3H), 4.10 – 4.01 (m, 1H), 2.53 (t, *J* = 8.0 Hz, 2H), 2.12 – 1.88 (m, 2H) ppm; ¹³C NMR (100 MHz, DMSO-*d*₆) δ 173.6, 156.1, 143.9, 140.7, 127.6, 127.1, 125.4, 120.1, 65.8, 53.3, 48.0, 46.6, 27.1 ppm; ESI-MS calcd for C₁₉H₂₀NO₇S (M+H)⁺: 406.1, found: 406.2.

2.2 Fmoc-His(N(π)-(CH₂)₈Ph, N(τ)-(CH₂)₂Ot-Bu)-OH (SI-5).



2.2.1 Fmoc-His[N(π)-(CH₂)₈Ph]-Oallyl (SI-4).

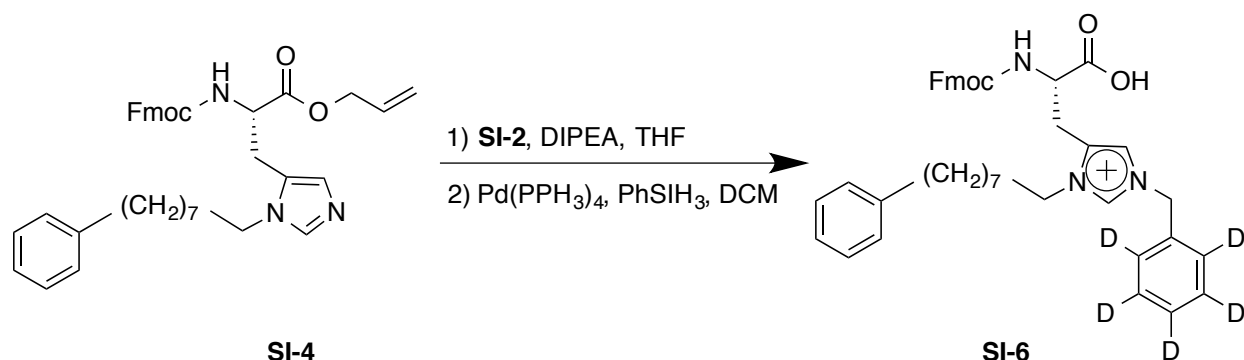
Diisopropyl azodicarboxylate (DIAD) (0.7 mL, 3.5 mmol) was added to a solution of triphenylphosphine (PPh₃) (0.9 g, 3.5 mmol) in dry THF (15 mL) at 0 °C under dry nitrogen in a flame-dried round-bottomed flask. The mixture was stirred until a white solid appeared and then stirring was continued (0 °C, 10 minutes). To the mixture was added allyl alcohol (0.24 mL, 3.5 mmol) and stirring was continued (45 minutes) and then **SI-1**

(1.0 g, 1.8 mmol) dissolved in THF (3 mL) was added and stirring was continued (3 h). Diethyl ether was added, and the mixture was washed (H₂O) and the combined organic layer was dried (MgSO₄), concentrated *in vacuo* and the product was purified by silica gel chromatography (TLC CHCl₃ : MeOH = 98 : 2) to provide **SI-4** 0.86g (80%). [α]_D²⁰ 1.2 (c 1.45, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.58 (s, 1H), 7.75 (d, *J* = 4.0 Hz, 2H), 7.57 (d, *J* = 8.0 Hz, 2H), 7.42 – 7.14 (m, 10H), 6.05 (d, *J* = 8.0 Hz, 1H), 5.94 – 5.80 (m, 1H), 5.38 – 5.24 (m, 2H), 4.71 – 4.51 (m, 3H), 4.37 (d, *J* = 8.0 Hz, 2H), 4.18 (t, *J* = 8.0 Hz, 1H), 4.07 – 3.94 (m, 2H), 3.31 – 3.11 (m, 2H), 2.58 (t, *J* = 8.0 Hz, 2H), 1.76 (s, 2H), 1.63 – 1.53 (m, 2H), 1.28 (s, 8H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 169.9, 156.2, 143.74, 143.69, 142.9, 135.0, 131.0, 129.4, 128.6, 128.4, 128.0, 127.3, 125.8, 125.2, 120.3, 120.2, 119.4, 67.5, 67.1, 53.1, 47.2, 47.0, 36.0, 31.5, 30.3, 29.4, 29.2, 29.0, 26.5, 26.4 ppm; ESI-MS calcd for C₃₈H₄₄N₃O₄ (M+)⁺: 606.3, found: 606.5.

2.2.2 Fmoc-His(N(π)-(CH₂)₈Ph, N(τ)-(CH₂)₂Ot-Bu)-OH (SI-5).

To a stirred solution of triflic anhydride (1.0 M in CH₂Cl₂, 0.73 mL) in CH₂Cl₂ (2.3 mL) under nitrogen at -75 °C was added a solution of ethylene glycol mono-*tert*-butyl ether (96 μ L, 0.73 mmol) and diisopropylethylamine (DIPEA) (127 μ L, 0.73 mmol) in CH₂Cl₂ (1.6 mL) dropwise over 10 minutes and stirring was continued at -75 °C (20 minutes). A solution of **SI-4** (0.4 g, 0.66 mmol) in CH₂Cl₂ (2.7 mL) was added drop-wise and the reaction mixture was allowed to gradually warm to room temperature and stirred (overnight). The mixture was poured into aqueous NaHCO₃ and stirred vigorously (30 minutes). The organic layer was diluted (CH₂Cl₂), washed (aqueous NaHCO₃ and brine), dried (Na₂SO₄) and evaporated to a gum. Purification by silica gel flash chromatograph (CHCl₃ : MeOH = 98 : 2) to provide the intermediate allyl ester (0.35g, 75%). A mixture of this material (0.3 g, 0.42 mmol), Pd(PPh₃)₄ (49 mg, 42 μ mol) and phenylsilane (79 μ L, 0.64 mmol) in CH₂Cl₂ (4.2 mL) was stirred under nitrogen (30 minutes). The crude product was concentrated and purified by silica gel chromatography (CHCl₃ : MeOH from 98 : 2 to 9 : 1) to provide **SI-5** (0.24 g; 85%). [α]_D¹⁹ 21.5 (c 1.3, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.54 (s, 1H), 7.75 (d, *J* = 8.0 Hz, 2H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.46 – 7.11 (m, 10H), 6.64 (d, *J* = 8.0 Hz, 1H), 6.23 (brs, 1H), 4.65 – 4.53 (m, 1H), 4.40 – 3.91 (m, 7H), 3.55 (s, 2H), 3.27 (s, 2H), 2.54 (t, *J* = 8.0 Hz, 2H), 1.74 (s, 2H), 1.54 (s, 2H), 1.23 (s, 9H), 1.06 (s, 8H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 172.0, 156.4, 144.0, 143.7, 142.9, 141.4, 135.6, 130.5, 128.6, 128.4, 128.0, 127.4, 125.8, 125.6, 125.3, 122.0, 120.2, 74.1, 67.6, 59.8, 53.4, 50.8, 47.4, 47.1, 36.0, 31.6, 30.1, 29.4, 29.3, 29.1, 27.4, 26.4, 26.3 ppm; ESI-MS calcd for C₄₁H₅₂N₃O₅ M⁺: 666.4, found: 666.6.

2.3 Fmoc-His(N(π)-(CH₂)₈Ph, N(τ)-d₅-Bn)-OH (SI-6).

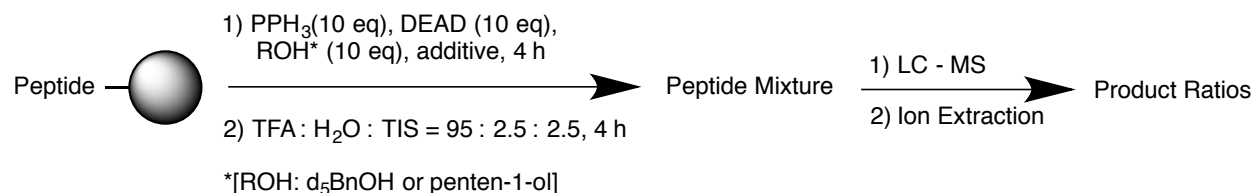


To a solution of **SI-4** (0.2 g, 0.33 mmol) in THF (3.3 mL) was added **SI-2** (0.15 g, 0.66 mmol) and DIPEA (0.12 mL, 0.66 mmol). The mixture was stirred (overnight) and then concentrated and purified by silica gel column chromatography (CHCl₃ : MeOH, 98 : 2) to provide the intermediate allyl ester (0.18 g, 78%). A solution of this material (0.18 g, 0.26 mmol) with Pd(PPh₃)₄ (59 mg, 0.026 mmol) and dimedone (42 mg, 0.38 mmol) in CH₂Cl₂ (2.6 mL) was stirred under nitrogen (30 minutes) and the mixture was concentrated and purified by silica gel column chromatography (CHCl₃ : MeOH from 98 : 2 to 9 : 1) to provide **SI-6** (0.13 g, 77%). [α]_D²⁰ 29.0 (c 2.35, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 9.58 (s, 1H), 7.71 (s, 2H), 7.62 – 7.50 (m, 2H), 7.40 – 7.30 (m, 2H), 7.29 – 7.19 (m, 4H), 7.19 – 7.08 (m, 4H), 6.62 (s, 1H), 5.41 – 5.09 (m, 2H), 4.41 – 3.87 (m, 6H), 3.23 (t, *J* = 4.0 Hz, 2H), 2.52 (t, *J* = 8.0 Hz, 2H), 1.67 (s, 2H), 1.52 (s, 2H), 1.18 (s, 8H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 173.5, 156.3, 144.2, 143.9, 142.9, 141.43, 141.37, 135.5, 133.5, 133.0, 128.5, 128.4, 127.9, 127.2, 125.8, 125.4, 125.3, 120.5, 120.1, 66.8, 55.1, 52.8, 47.3, 36.0, 31.6, 30.4, 29.9, 29.4, 29.3, 29.1, 26.7, 26.5 ppm; ESI-MS calcd for C₄₂H₄₁D₅N₃O₄ M⁺: 661.4, found: 661.6.

3. Showing the Importance of the pThr Residue

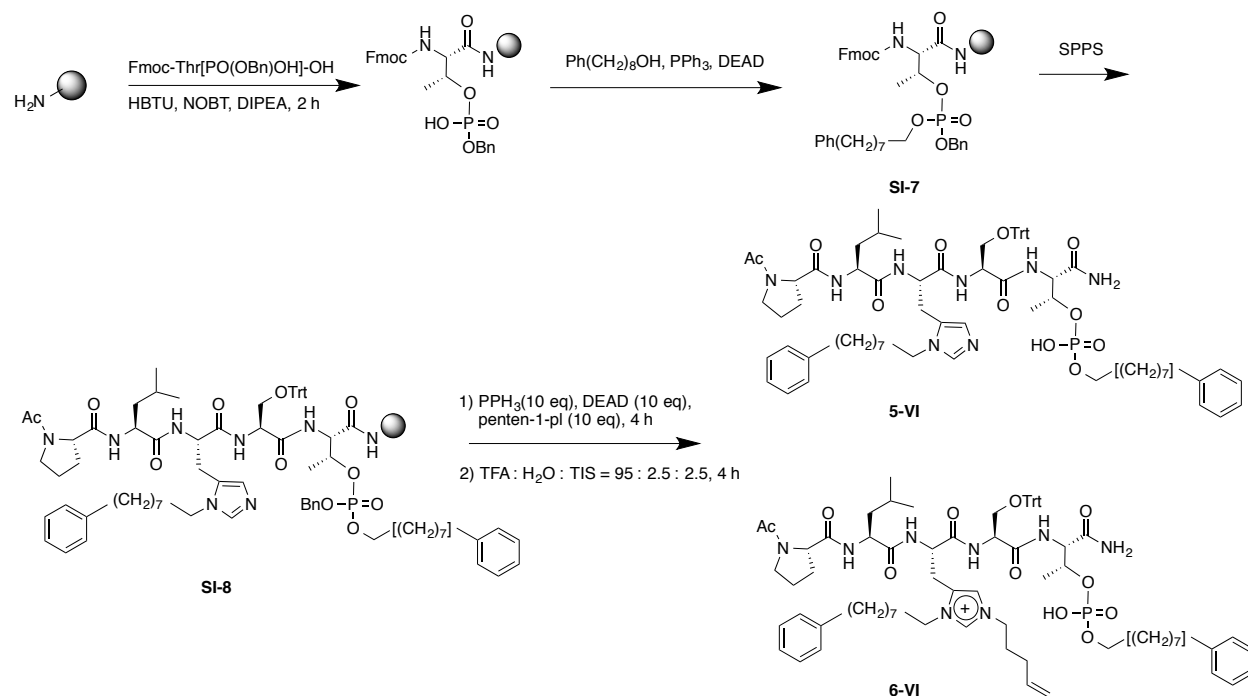
3.1 General Peptide Synthesis Procedures. Resin bound peptides were synthesized on NovaSyn®TGR resin following standard Fmoc-based solid-phase protocols using *N*-methyl-2-pyrrolidone (NMP) as solvent. 1-*O*-Benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate (HBTU) (5.0 equivalents), hydroxybenzotriazole (HOBT) (5.0 equiv) and *N,N*-diisopropylethylamine (DIPEA) (10.0 equivalents) were used as coupling reagents (2 h). Removal of Fmoc protection was conducted using 20% piperidine in DMF (20 minutes). Amino terminal acetylation was achieved using 1-acetylimidazole (10 equivalents) in DMF (4 h).

3.2 On-resin Mitsunobu Reactions and Analytical LC-MS Analysis.



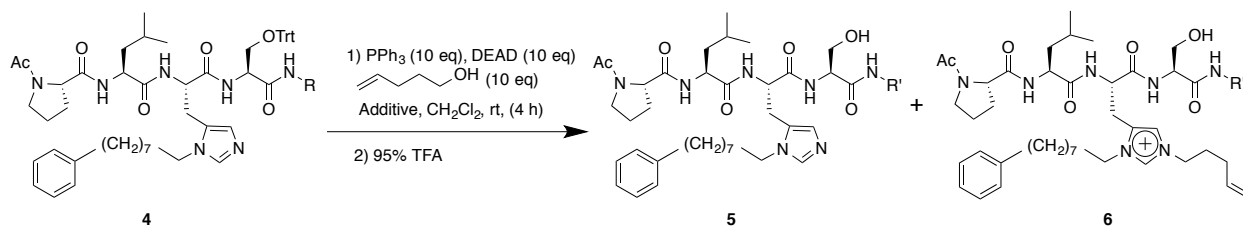
Resin-bound peptides were treated with PPh_3 (10.0 eq.), DEAD (10.0 equivalents) and alcohol (10.0 equivalents) and additive as indicated in Table S1 in dry CH_2Cl_2 (4 mL) at room temperature (4 h). The resulting resin-bound peptides were washed sequentially with DMF, MeOH, CH_2Cl_2 and Et_2O and dried under vacuum. The resins (0.1 mmol) were cleaved by treating with TFA : H_2O : triisopropylsilane (95: 2.5: 2.5, 4 mL) (4 h). The cleavage mixtures were filtered and filtrates were concentrated. The crude products were dissolved in MeCN : H_2O (v/v 1:1 with 0.1% TFA) to achieve a final 100 μM concentration and a 5 μL aliquots were subjected to LC-MS analyses [column: Zorbax ODS (100 mm x 2.1 mm); A = H_2O + 0.1% acetic acid, B = MeOH + 0.1% acetic acid; Gradient conditions: B% isocratic at 5% (3 minutes); from 5% to 90% over 8 minutes; isocratic at 90% for 6 minutes, then decrease from 90% to 5% over 5 minutes]. Ions for the desired products were extracted from total ion spectra and the peak areas were used to determine product ratios. Ratios of products **5** and **6** are shown in Table S3.

3.3 Synthesis of Resin-bound Peptide SI-8 for Experiment 3.VI of Table S3.

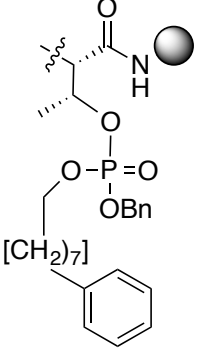
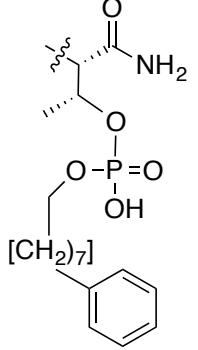


Coupling of the pThr residue to the resin was accomplished using HBTU (5.0 equivalents), HOBT (5.0 equivalents) and DIPEA (10.0 equivalents) in NMP (2 h). The resulting resin was treated with 8-phenyl-1-octanol (10.0 equivalents), PPh_3 (10.0 eq.), DEAD (10.0 equivalents) in CH_2Cl_2 (4 h) to yield resin **SI-7**. Coupling of the remaining residues using standard protocols outlined above afforded resin **SI-8**. Mitsunobu coupling with penten-1-ol as outlined above, followed by resin cleavage yielded peptides **5-VI** and **6-VI** in the ratios indicated in Experiment 3.VI of Table S3.

Table S3. Examination of the Role of the pThr Residue in Mitsunobu-induced N(τ)-Alkylation.

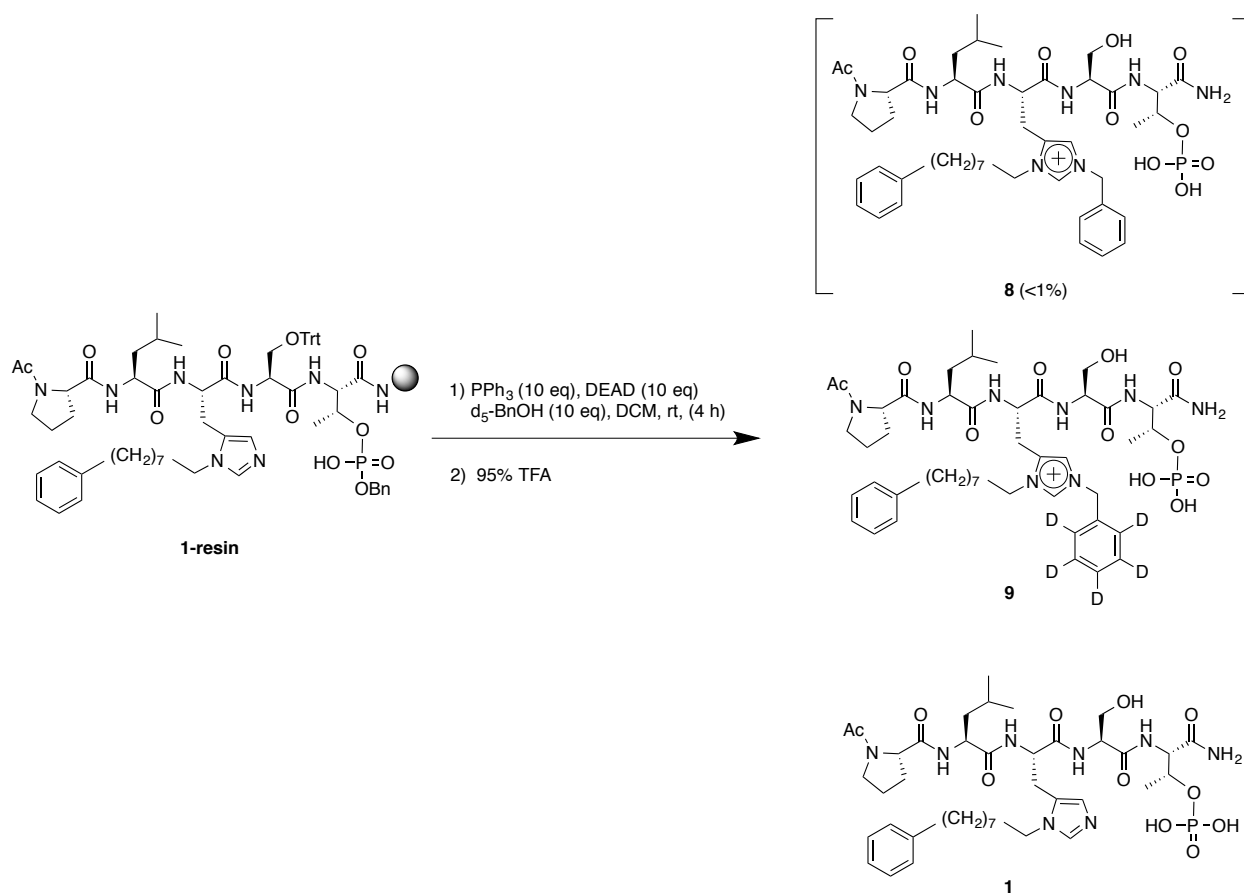


Experiment	R	R'	Additive	Product Ratio (5 : 6)
3.I		H	None	100 : 0 ^a
3.II		H		31 : 1
3.III		H		30 : 1
3.IV		H		30 : 1
3.V		H		16 : 1

3.VI ^b			None	5-VI : 6-VI 7 : 1
-------------------	---	---	------	------------------------------------

^a No **6** detectable. ^bResin **SI-8** used.

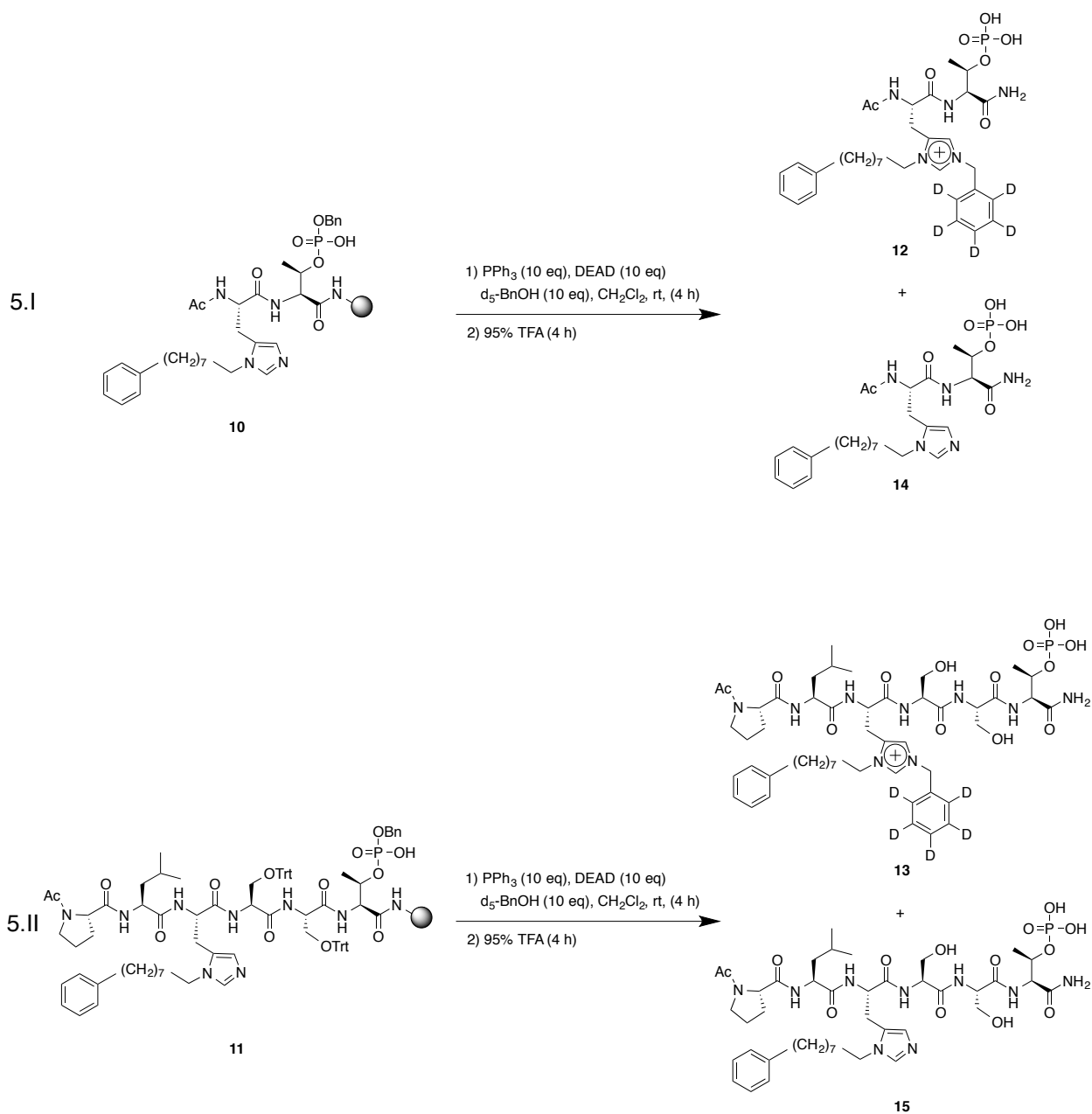
4. Showing that Phosphoryl Ester Transfer is not Involved



Mitsunobu treatment of **1-resin** as outlined in Section 3.2 was performed using $\text{d}_5\text{-BnOH}$ as the alcohol source. Following resin cleavage (95% TFA) product mixtures were analyzed by

HPLC – mass spec as outlined in Section 3.2. Extracted ion spectra showed that <1% of product exhibited a molecular weight consistent with peptide having a CH₅CH₂-adduct (peptide **8**). The ratio of N(τ)-CH₂CD₅ containing peptide (**9**) to non-alkylated peptide (**1**) was found to be: (**9** : **1** ≈ 9 : 1). This indicates that initial phosphoryl esterification with d₅-BnOH followed by intramolecular transfer of phosphoryl ester to the N(τ)-position had not occurred to any appreciable extent.

5. Showing that N(τ)-Alkylation can Accommodate Spatial Differences between the pThr and His Residues



Experiments **5.I** and **5.II** (outlined above) were undertaken to examine the effects of varying the spatial separation between the pThr residue and the His* residue. On-resin Mitsunobu treatment of a peptide lacking a Ser residue between the pThr and His* residues (resin **10**, Experiment **I**) was performed as outlined in Section 3.2 using d₅-BnOH as the alcohol source. Following resin cleavage (95% TFA) the ratio of N(τ)-CH₆CD₅ containing peptide (**12**) to non-alkylated peptide (**14**) were determined from the extracted ion spectra following HPLC-mass spec analysis. Analysis of a typical experiment is shown below:

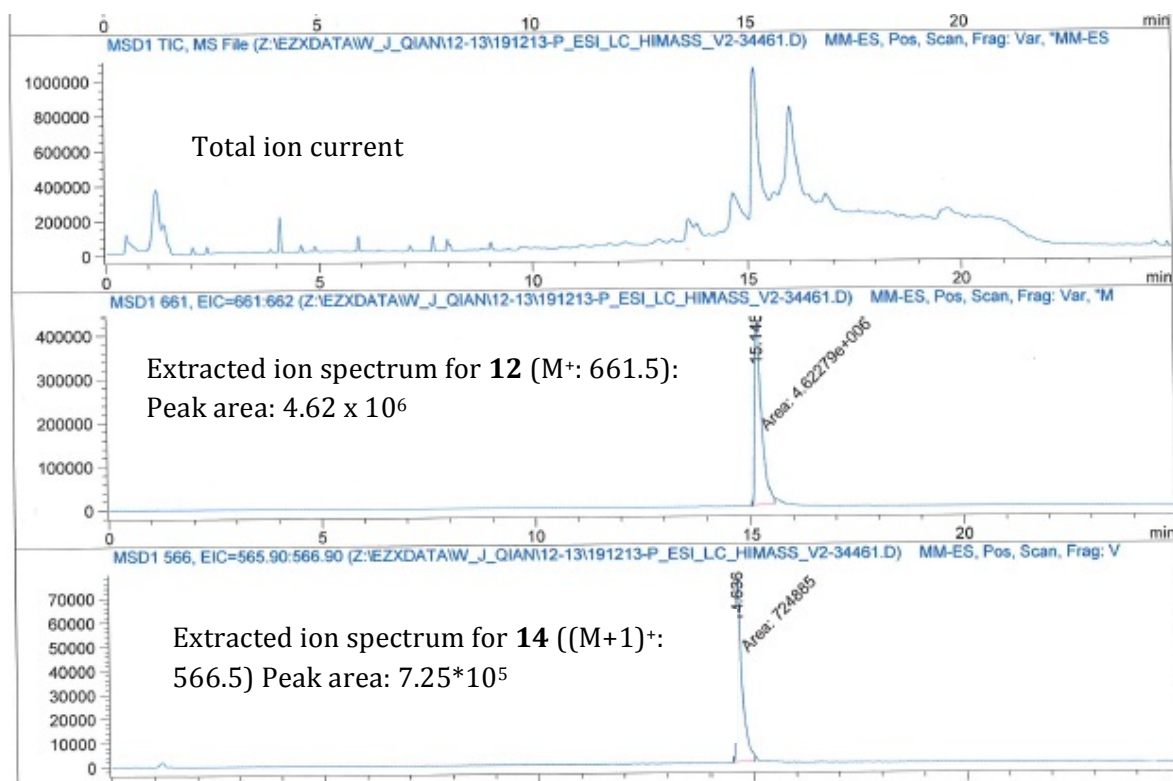


Figure S5. HPLC-mass spectral analysis of Mitsunobu reaction obtained from Experiment 5.I (above). A 5 μ L aliquot of the crude product in MeCN : H₂O (v/v) 1 : 1 with 0.1% TFA) to achieve 100 μ M concentration was subjected to HPLC-mass spectral analysis using a Zorbax-C₁₈ column(100 x 2.1 mm); A = H₂O + 0.1% acetic acid, B=methanol + 0.1% acetic acid, Gradient: B%: isocratic 5% (3 minutes); linear gradient from 5% to 90% over 8 minutes; isocratic 90% for 6 minutes decrease 90% to 5% over 5 minutes.

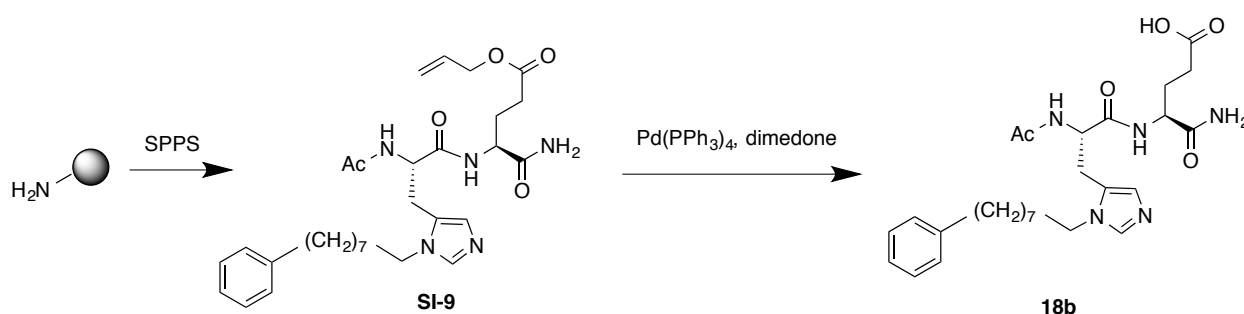
These analyses showed that for Experiment **5.I**, the ratio of N(τ)-alkylated to non-alkylated products (**12** to **14**, respectively) was approximately (6 : 1), while for Experiment **II**, the ratio of N(τ)-alkylated to non-alkylated products (**13** to **15**, respectively) was approximately (7 : 1).

6. Evidence that N(τ)-Alkylation can be Promoted by Residues other than pThr

6.1 General Synthesis of Peptides for Table S6.1.

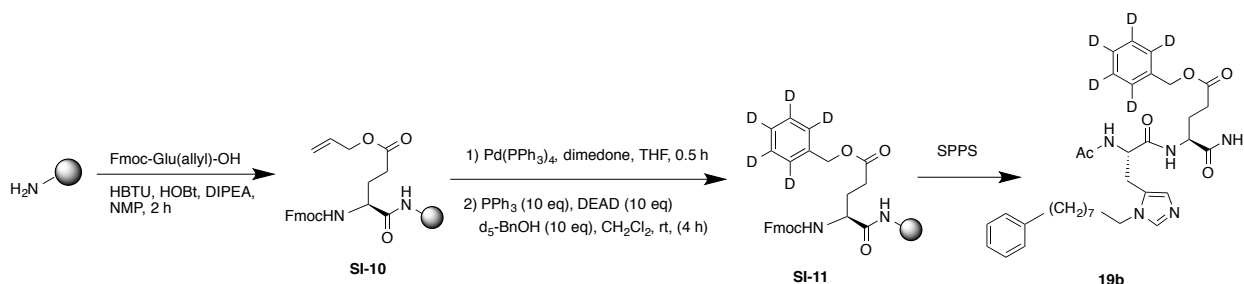
In order to examine whether acidic residues other than pThr could promote on-resin Mitsunobu His* N(τ)-alkylation, experiments 6.1 – 6.7 were performed on resins **18a** – **18f** (Table S6). Experimental conditions were as outlined in Section 3.2 using d_5 -BnOH as the alcohol source. Following resin cleavage (95% TFA) the ratio of N(τ)-CH₂CD₅ containing peptide (**19**) to non-alkylated peptide (**20**) were determined from the extracted ion spectra following HPLC-mass spec analysis. Data are shown in Table S6.1.

6.2 Synthesis of Resin-bound Peptide **18b** for Experiment 6.2 of Table S6.



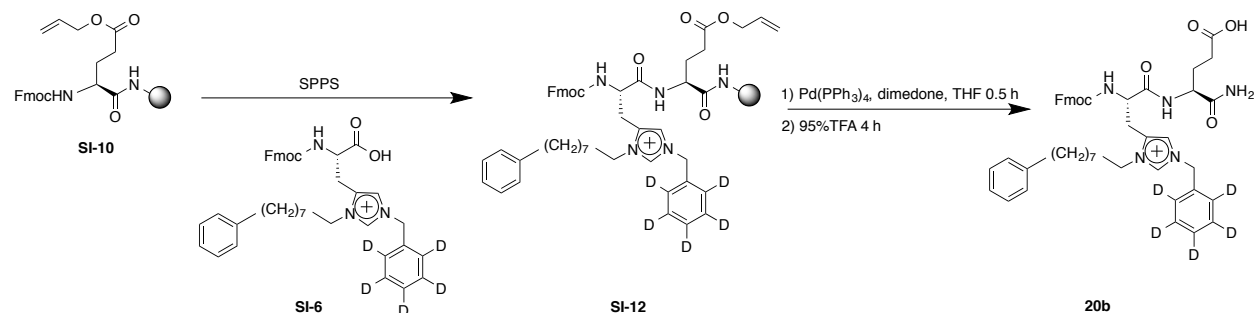
Resin **SI-9** was treated with Pd(PPh₃)₄ (0.1 equivalents) and dimedone (2.0 equivalents) in THF (30 minutes) to afford resin **18b**.

6.3 Verification of the Structure of Peptide **19b** from Experiment 6.2 of Table S6.



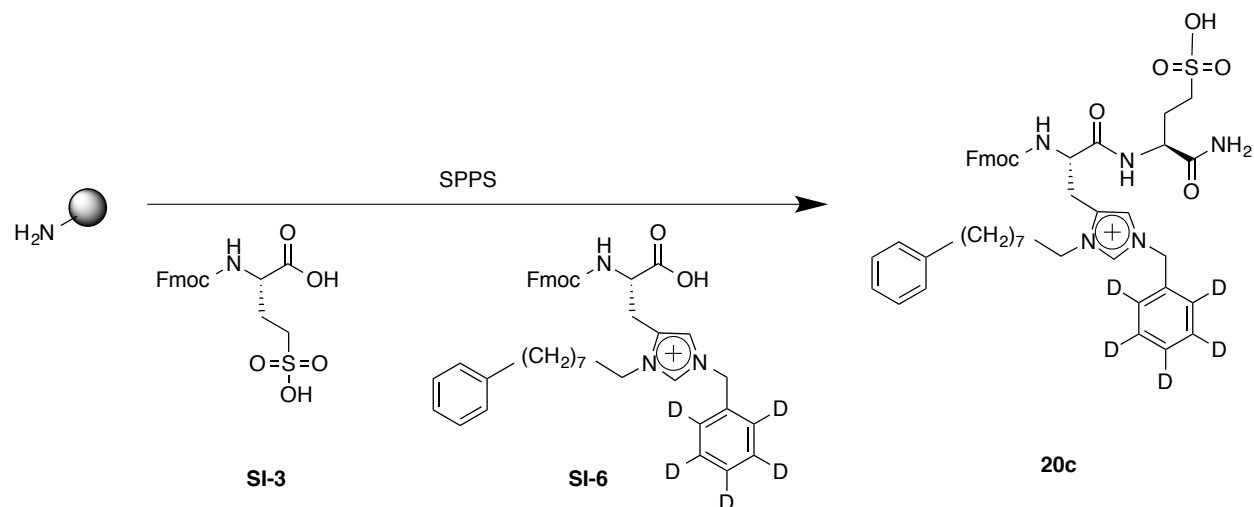
Coupling of the Fmoc-Glu(allyl)-OH residue to the resin was accomplished using HBTU (5.0 equivalents), HOBT (5.0 equivalents) and DIPEA (10.0 equivalents) in NMP (2 h). The allyl ester of resin **SI-10** was de-protected by treating with Pd(PPh₃)₄ (0.1 equivalents) and dimedone (2.0 equivalents) in THF for (30 minutes) and the resin was then treated with d_5 -BnOH (10.0 equivalents) PPh₃ (10.0 equivalents) and DEAD (10.0 equivalents) in CH₂Cl₂ (4 h) to afford resin **SI-11**. Histidine analog **SI-1** was then incorporated under standard conditions to provide peptide **19b** following resin cleavage. This served as an authentic reference for product identification in Experiment 6.2 of Table S6 (HPLC – mass spec retention time of 15.8 minutes, with co-injection providing a single peak).

6.4 Verification of the Structure of Peptide 20b from Experiment 6.2 of Table S6.



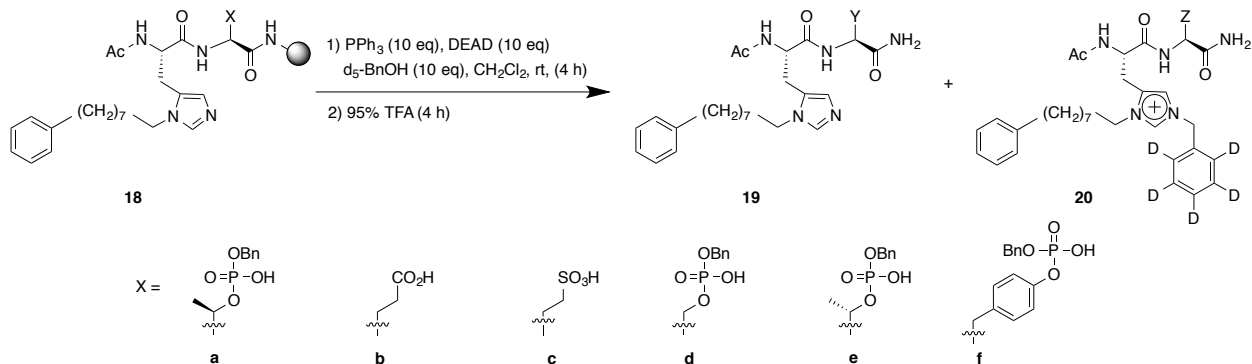
Histidine analogue **SI-6** was coupled to resin **SI-10** utilized to prepare resin **SI-12**. The allyl ester of **SI-12** was removed by treating with Pd(PPh₃)₄ (0.1 equivalents) and dimedone (2.0 equivalents) in THF for (30 minutes) and the resin was cleaved (95% TFA, 4 h) to afford **20b** as an authentic reference for product identification in Experiment 6.2 of Table S6 (HPLC – mass spec retention time of 15.4 minutes, with co-injection providing a single peak).

6.5 Verification of the Structure of Peptide 20c from Experiment 6.3 of Table S6.

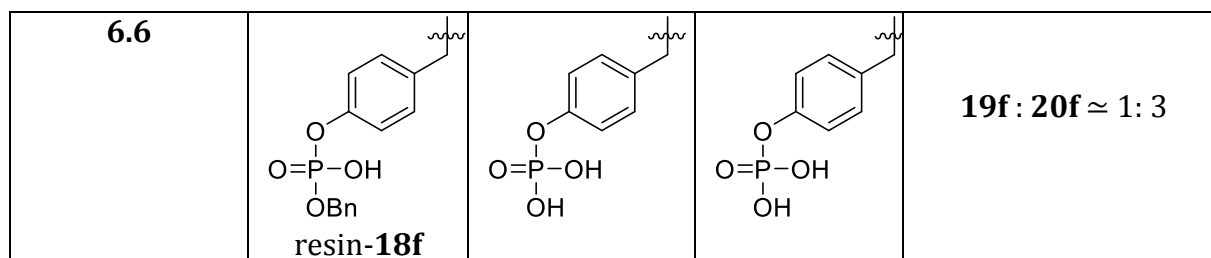


Dipeptide **20c** was synthesized by standard protocols outlined above utilizing reagents **SI-3** (prepared in Section 2.1) and **SI-6** (prepared in Section 2.3). The product served as an authentic reference for peptide **20c** of Experiment 6.3 in Table S6 (HPLC – mass spec co-elution of 15.1 minutes).

Table S6. Examination of On-resin Mitsunobu N(τ)-Alkylation on Peptides Having Differing Acidic Residues.

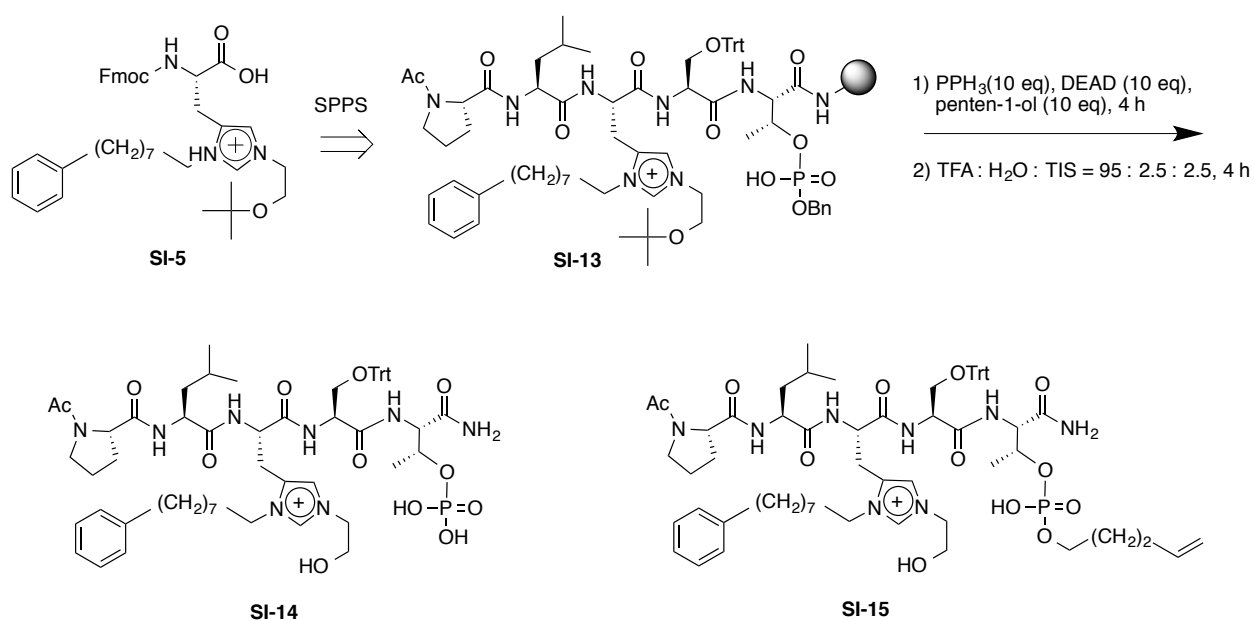


Experiment	X =	Y =	Z =	Product Ratios ^a
6.1	 resin-18a	 resin-18a	 resin-18a	19a : 20a \approx 1 : 6
6.2	 resin-18b	 resin-18d	 resin-18e	19b : 20b \approx 3 : 1
6.3	 resin-18c	 resin-18c	 resin-18c	19c : 20c \approx 1 : 70
6.4	 resin-18d	 resin-18d	 resin-18d	19d : 20d \approx 1 : 3
6.5	 resin-18e	 resin-18e	 resin-18e	19e : 20e \approx 1 : 2



^aRatio determined by HPLC-MS ion-extracted analysis using a Zorbax-C₁₈ column(100 x 2.1 mm); A = H₂O + 0.1% acetic acid, B=methanol + 0.1% acetic acid, Gradient: B%: isocratic 5% (3 minutes); linear gradient from 5% to 90% over 8 minutes; isocratic 90% for 6 minutes decrease 90% to 5% over 5 minutes.

7. Showing that N(π), N(τ)-bis-alkylation Inhibits Mitsunobu Esterification of a Proximal pThr[(OBnO)(OH)] Residue.



In order to examine the effects of N(π), N(τ)-bis-alkylation on Mitsunobu esterification of a proximal pThr[(OBnO)(OH)] residue we employed reagent **SI-5** (prepared in Section 2.2) to construct resin **SI-13**, which contained a His* residue having a *tert*-butyl-protected 2-hydroxyethyl group at the N(τ)-position. We then subjected this resin to Mitsunobu coupling with 10 equivalents of penten-1-ol as outlined in Section 3.2. When we examined the product mixture using HPLC – mass spec ion-extracted chromatography, we found that the ratio of esterified to non-esterified peptides was **SI-14** : **SI-15** \approx 15 : 1.

8. Mass Spectral Characterization

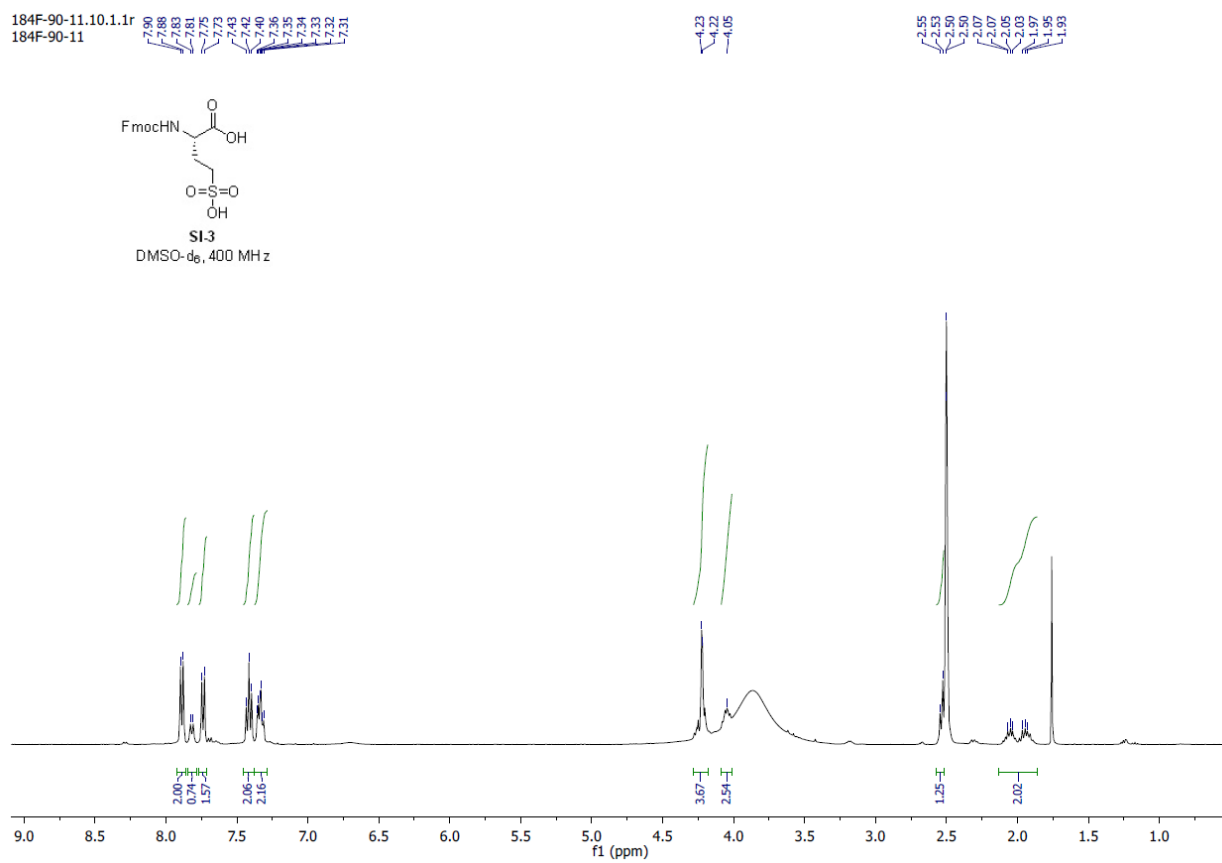
Table S8. Electrospray Ionization (ESI) Mass Spectral Data.

No.	Expected (M + H) ⁺	Observed (M + H) ⁺
5	682.4	682.6
5-VI	1051.6	1051.8
6	750.5	750.6
6-VI	1119.7	1119.9
8	953.5	953.6
9	958.5	958.7
12	661.4	661.5
13	1045.6	1045.8
14	566.3	566.4
15	950.5	950.7
19b	609.4	609.5
19c	550.3	550.5
19d	552.2	552.4
19e	566.3	566.4
19f	628.3	628.5
20b	609.4	609.5
20c	645.4	645.5
20d	647.3	647.5
20e	661.4	661.5
20f	723.4	723.6
SI-14	907.5	907.7
SI-15	975.5	975.7

9. References.

1. Qian, W.; Liu, F.; Burke, T. R. Jr. *J. Org. Chem.* **2011**, 76, 8885.
2. Li, D.-P.; Pan, X.-Q.; An, L.-T.; Zou, J.-P.; Zhang, W. *J. Org. Chem.* **2014**, 79, 1850.

10. ¹H-NMR and ¹³C-NMR spectra for SI-3 – SI6.



184F-90-15C.10.1.1r
184F-90-15C

156.11

143.89

140.71

127.65

127.14

125.41

120.12

65.76

53.34

48.04

46.63

40.13

39.92

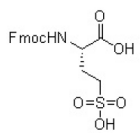
39.51

39.30

39.10

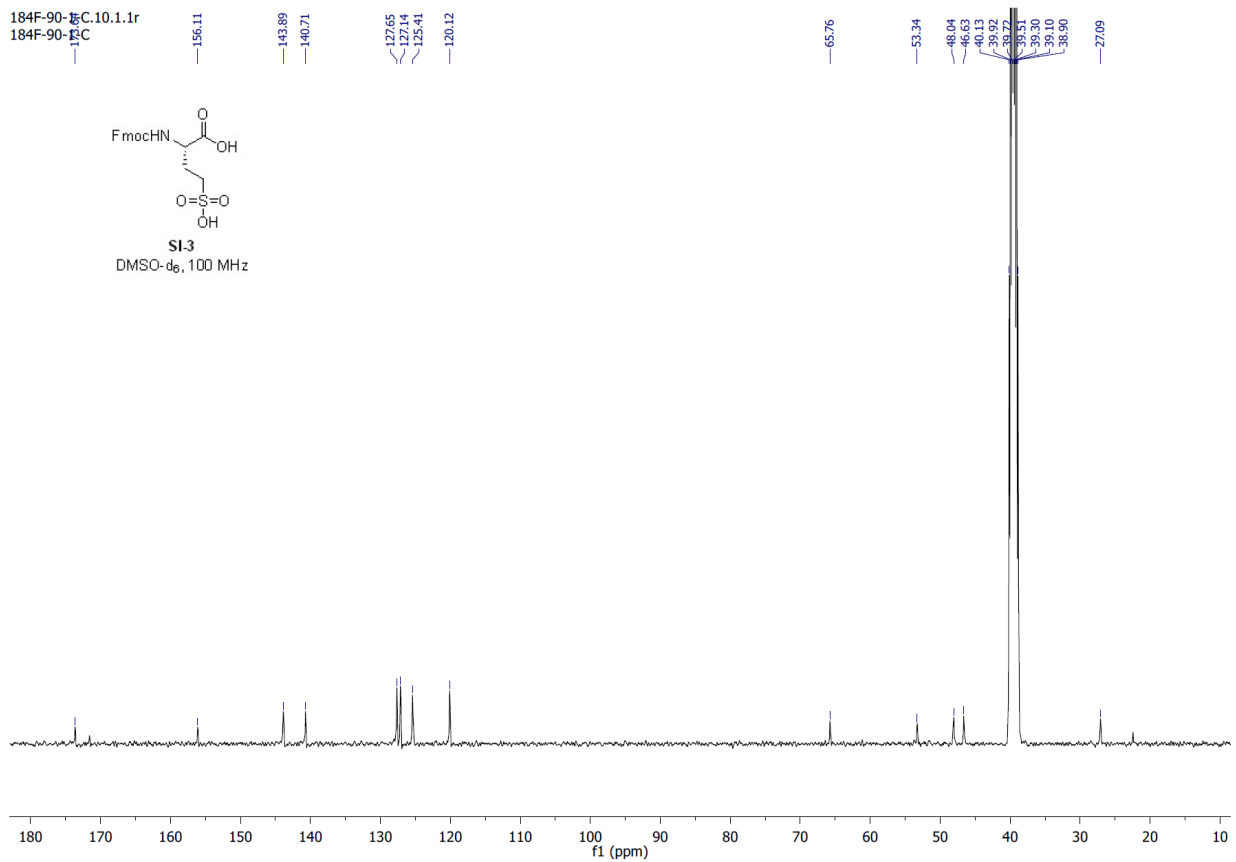
38.90

27.09

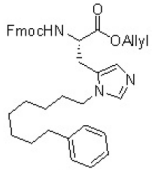


S1-3

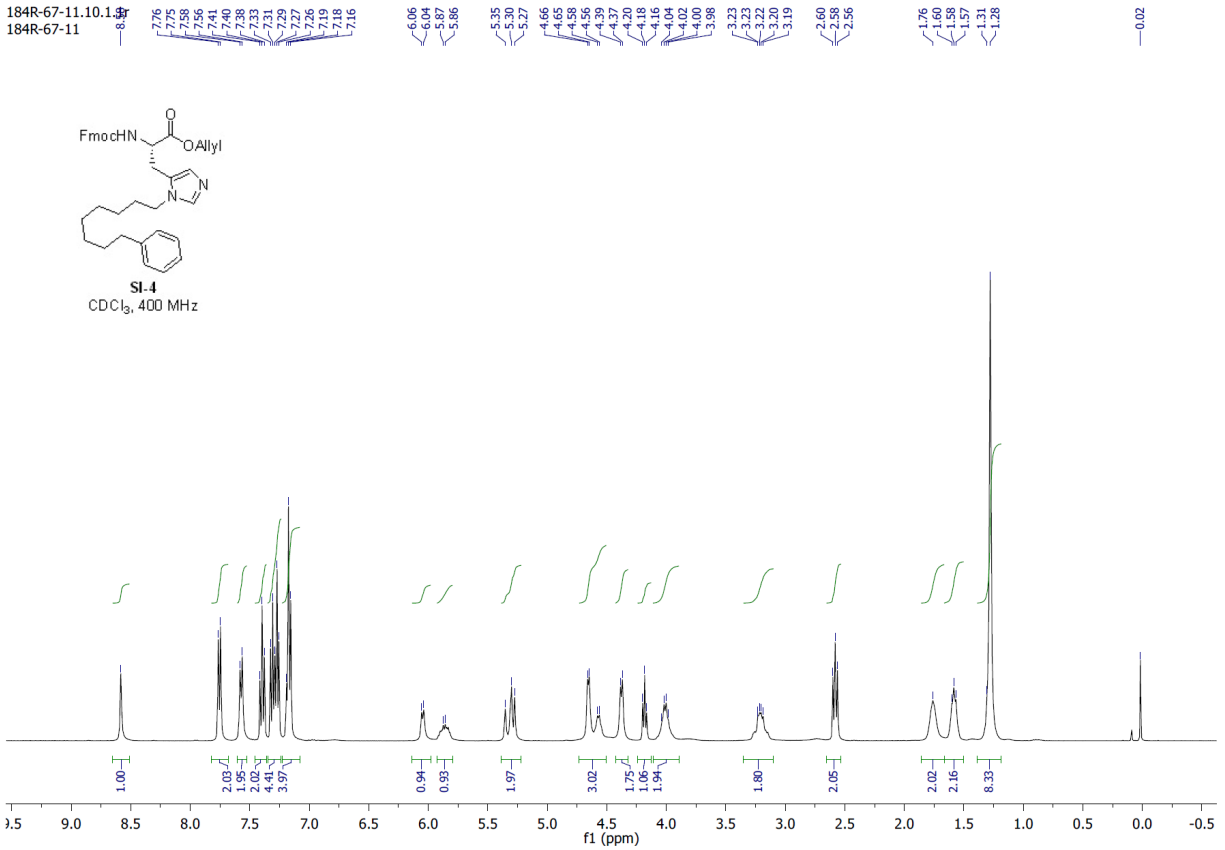
DMSO-d₆, 100 MHz



184R-67-11.10.1
184R-67-11



SI-4
CDCl₃, 400 MHz

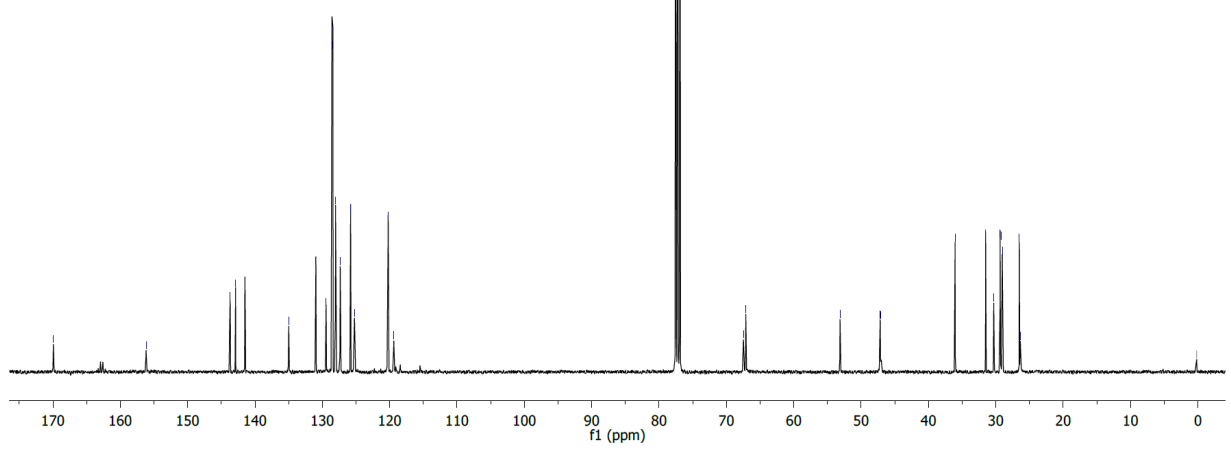
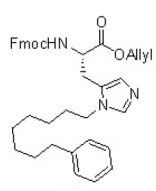


184R-67-11-C.10.1.1r
184R-67-11-C

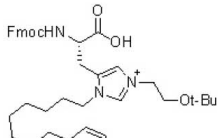
169.93
156.17
143.74
143.69
142.88
138.98
138.86
129.45
128.57
128.43
128.04
127.34
125.80
125.72
120.21
120.23
119.39

77.55 CDCl₃
77.49 CDCl₃
77.03 CDCl₃
76.91 CDCl₃
67.48
67.12
53.13
47.18
47.05
36.05
31.52
30.31
29.37
29.24
29.04
26.51
26.36

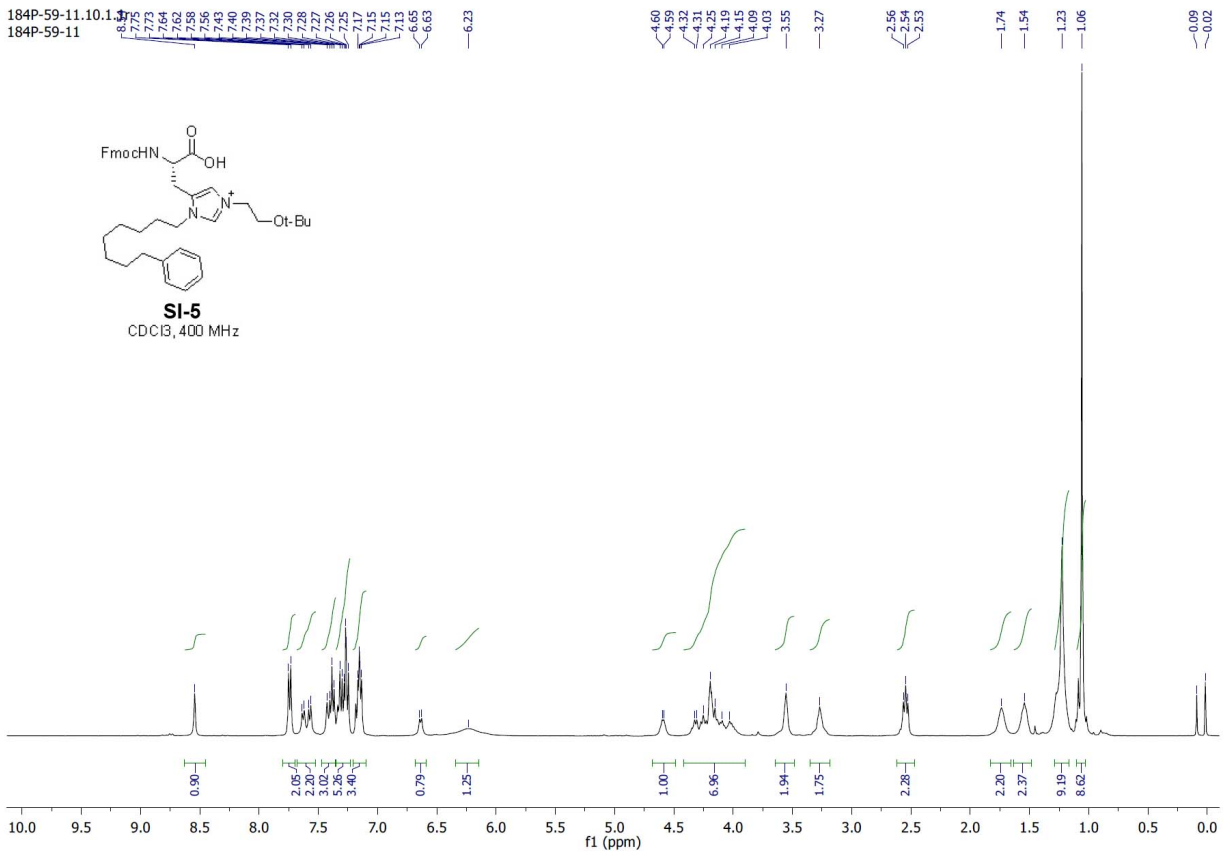
0.19



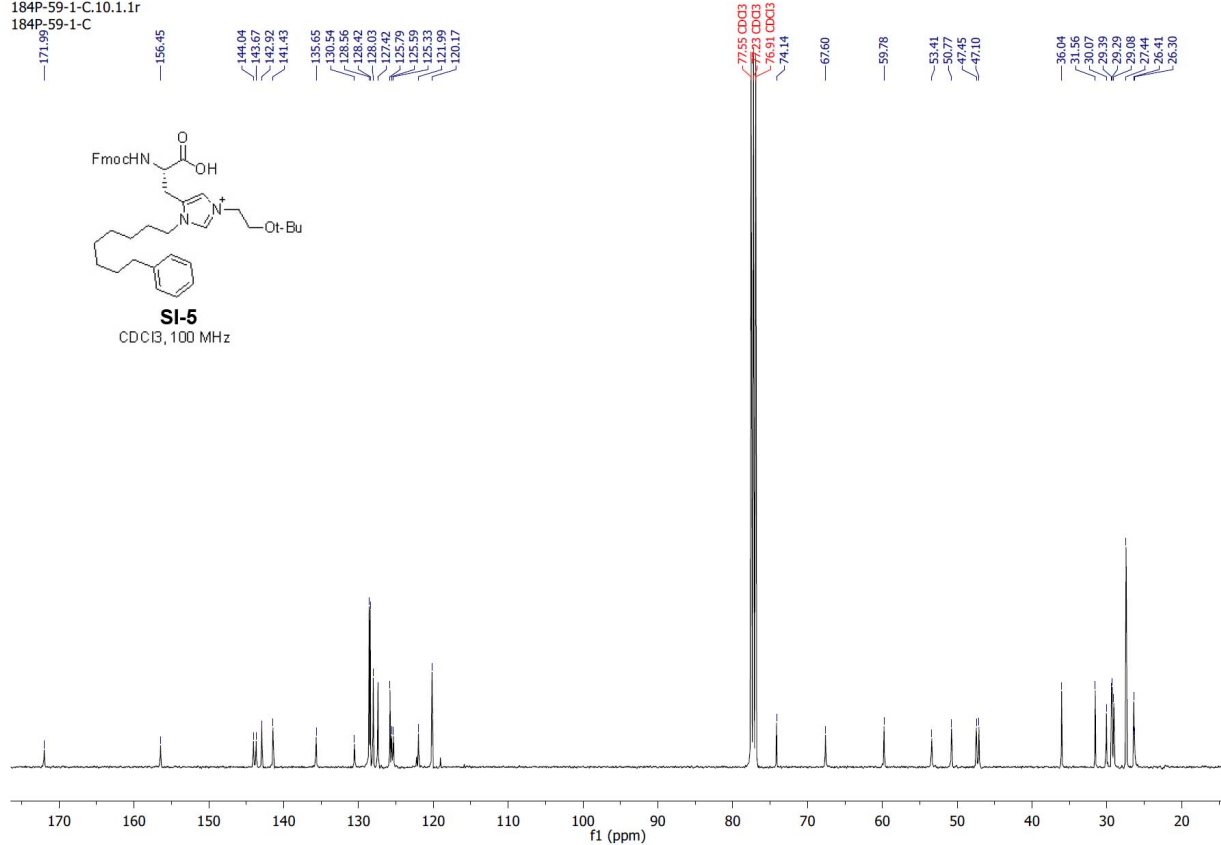
184P-59-11.10.1
184P-59-11



SI-5
CDCl₃, 400 MHz



184P-59-1-C.10.1.1r
184P-59-1-C



184S-57-1.10.1.10
184S-57-1

7.71
7.59
7.57
7.54
7.54
7.38
7.34
7.32
7.27
7.25
7.23
7.17
7.16
7.14
7.12
6.62

5.32
5.28
5.22
5.18

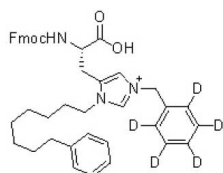
4.27
4.24
4.13
4.12

3.23

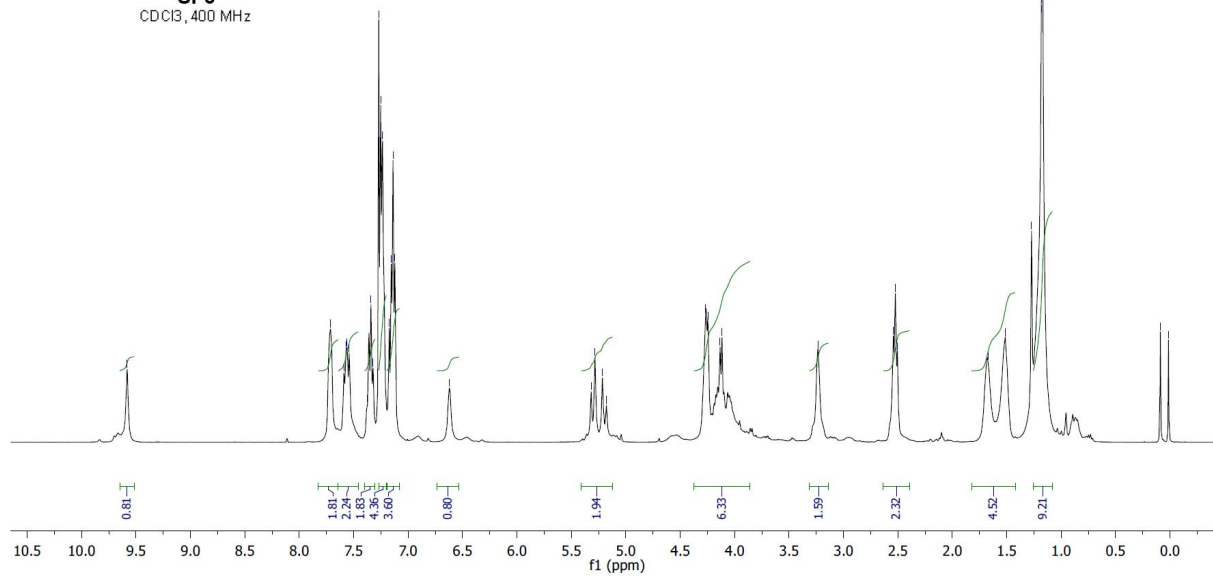
2.54
2.52
2.50

1.67
1.52
1.27
1.18

0.09
0.01



SI-6
CDCl₃, 400 MHz



184S-57-1-C.10.1.1r
184S-57-1-C

